

Physiological Quality of Maize Hybrid Seeds Treated with Copper Nanoparticles

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Abstract— In corn, several factors determine productivity, especially those inherent in planting and the quality of seeds. Nanoparticles that contain some essential element in plants are classified as nanofertilizers, which are allegedly more efficient than traditional fertilizers. The experimental design used was the Randomized Block Design (DBC), in a factorial scheme (5 x 5) with 3 repetitions. The seeds of corn hybrids treated with copper nanoparticles were subjected to germination and seed vigor tests. The data were submitted to Analysis of Variance (ANOVA) by the F test ($p \leq 0.05$), for the nanocopper doses, regression analysis ($p \leq 0.05$) was performed with the choice of mathematical models through the coefficient of determination (R^2). Differences between means were compared using the Tukey test ($p \leq 0.05$). Seed vigor was assessed by testing the first count and germination speed. Corn hybrids showed an average of 95.6% in relation to normal plants, not expressing a significant difference between them. The results indicate an optimum value for the seed treatment time and for the concentration of copper nanoparticles in the reaction medium. The longer the seed treatment time and the higher the concentrations in the reaction medium, it is possible to incorporate higher amounts of nanoparticles to the seeds without the treatment. The presence of nanoparticles in the seeds did not affect the germination speed per hybrid. This indicator shows that nanoparticles do not interfere with metabolic mechanisms in the germination phase.

Keywords— Corn hybrids, copper nanoparticles, physiological quality.

I. INTRODUCTION

Corn is characterized by being a crop with a low plant population, so production can be significantly compromised by being attacked by pests and diseases during the period of seed germination and seedling emergence. For this reason, it is extremely important that all the seeds sown germinate and ensure, the desired number of plants at the time of harvest and the good yield of the crop [1]. In the quest to raise current income levels and reduce corn production costs in Brazil, new technologies have been incorporated into production systems. Among these, we highlight the use of nanoparticles of nutrients applied via seed treatment (TS), which is considered a promising agronomic strategy, as it guarantees success in establishing the respective crop, enabling plants to have a greater capacity to resist biotic stresses (caused by pests and diseases) or abiotics

(depending on environmental conditions and nutrition) during the definition of the yield components.

In corn, several factors determine productivity, especially those inherent in planting and the quality of seeds. For example, if the physiological potential, represented by germination and vigor, determines the capacity of the seed to produce a normal seedling, that potential can be compromised in the production process because of factors such as genotype, in which cultivars of the same species can have vigor and different longevity. As well as adversities in the development of seeds (availability of water and nutrients, temperature, occurrence of diseases and insects, etc.) can affect their performance, the procedures adopted in harvesting, processing and storage significantly influence their physiological potential [2].

The growing need for food production and the risk of depleting the mineral reserves of fertilizers, are driving the

search for new products to optimize plant nutrition and reduce losses by leaching and volatilization, which make the production system more expensive, in addition to contaminating natural resources. The micromineral copper (Cu) nanoparticles have potential for use as fertilizers, as they present less solubilization than the conventional source, reducing losses due to fixation [3].

Nanoparticles that contain some essential element in plants are classified as nanofertilizers, which are allegedly more efficient than traditional fertilizers [4]. However, for use in agriculture it is essential to understand not only its efficiency as a fertilizer, but also the effects of these products on plants and their behavior. For Zhao et al. [5], the physiological responses of plants exposed to nanoparticles are often used to elucidate their effects on growth, development and toxicity [6].

The physiological process of germination and root growth are indicators of toxicity widely used in studies of the interaction between nanoparticles and plants [7]. For Pokhrel et al. [8], in addition to inhibiting the germination process, structural changes in primary cells and reduced root growth may occur after exposure to nanoparticles, an effect attributed to plant growth.

Thus, the present research is justified, with the objective of evaluating the physiological aspects in corn hybrids submitted to TS with Cu nanoparticles.

II. MATERIALS AND METHODS

The study was conducted in the Technology and Seed Production laboratory of the Agronomy Course at the University of the West of Santa Catarina in the municipality of São José do Cedro / SC.

The experimental design used was the Randomized Block Design (DBC), in a factorial scheme (5 x 5), and in factor A the corn hybrids were allocated (H1: 22S18 TOP2®; H2: 20A30 VIPTERA®; H3: 20A80 TOP2®; H4: 22S18 TOP3® and H5: 20A20 TOP2®) and in factor B, Cu nanoparticles doses were allocated via TS (D1: control; D2: 100.00 mg.L-1; D3: 300.00 mg.L-1; D4: 900.00 mg.L-1 and D5: 2700.00 mg.L-1), with 3 repetitions. The seeds of corn hybrids treated with copper nanoparticles, were obtained through a master's research in Technology and Innovation Management at the Community University of the Region of Chapecó - UNOCHAPECÓ, entitled "Agronomic aspects in corn hybrids submitted to seed treatment with Copper Nanoparticles"[9].

The seeds of corn hybrids treated with copper nanoparticles were subjected to germination and seed vigor tests on hydrated paper towel substrates (Germitest) with a

volume of solution equivalent to three times its mass. For each made roll, three sheets of paper towels were used. Seed vigor was assessed by testing the first count and germination speed. The germination test was conducted with four sub-samples of 50 seeds for each treatment, according to the criteria established in the Rules for Seed Analysis (Figures 1 and 2). The prepared rolls, with three sheets of paper towels, were placed in a B.O.D. (model MA 415), regulated to maintain a constant temperature of 25 ± 2 °C [10].

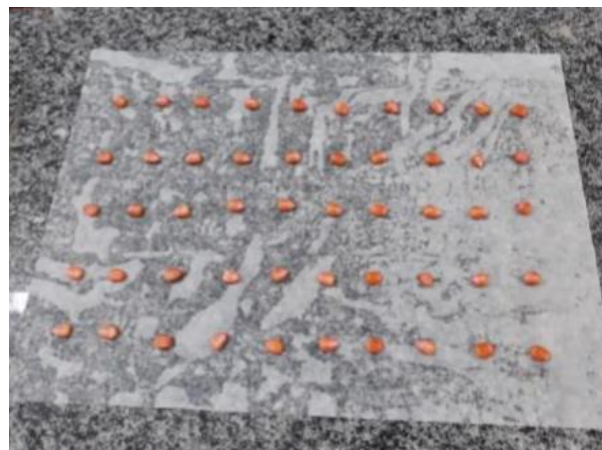


Fig.1 - Sub-sample of 50 seeds for each treatment of the experiment (São José do Cedro, SC - Safra 2018/2019)

Source: prepared by the author.



Fig.2 - Packaging of the sub-sample of 50 seeds for each treatment of the experiment (São José do Cedro, SC - Harvest 2018/2019)

Source: prepared by the author.

According to the same author, the evaluation of the first germination count was performed on the fourth day after the test was installed. The final germination count (second count), obtained by adding the first germination count, was performed on the seventh day after the test was

installed (Figure 3). The data were converted to percentage of normal seedlings.

From the first count and germination speed an indicator of the seed vigor was obtained and with the final count the viability. The germination speed was calculated using the Edmond and Drapala equation [11].



Fig.3 - Evaluation of the germination test for each treatment of the experiment (São José do Cedro, SC - Safra 2018/2019)

Source: prepared by the author.

The data collected were submitted to Analysis of Variance (ANOVA) by the F test ($p \leq 0.05$), for the nanocopper doses, regression analysis ($p \leq 0.05$) was performed with the choice of mathematical models through the coefficient of determination (R^2). Differences between means were compared using the Tukey test ($p \leq 0.05$). The computational application used was SISVAR - System of analysis of variance for balanced data [12].

III. RESULTS AND DISCUSSION

3.1 Percentage of normal seedlings

The analysis of variance revealed a significant effect ($p \leq 0.05$) of the factor of doses of Cu nanoparticles in relation to the variable response percentage of normal seedlings, that is, there is a mathematical model that explains the influence of variable X (doses of nanoparticles of Cu) in relation to variable Y (percentage of normal seedlings) (Figure 4).

As shown in figure 04, it can be seen that there was a cause and effect relationship between the variable nanoparticle doses and the percentage percentage of normal seedlings, that is, the doses of Cu nanoparticles influenced 96.93% in the percentage of normal seedlings, respectively, presenting a quadratic behavior.

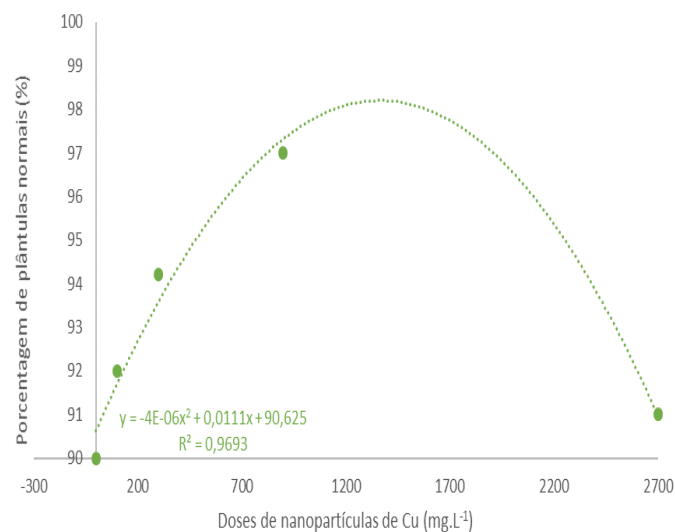


Fig.4 - Percentage of normal seedlings in the experiment in relation to the factor of doses of Cu nanoparticles (São José do Cedro, SC - Safra 2018/2019)

Source: prepared by the author.

In the present study, it can be noted that in relation to the growth of normal seedlings, the dose that showed the best results was 900mg / L-1 of nanocopper, influencing 96% in the seed germination of the corn hybrids. The increase in the number of normal seedlings can be considered an added benefit due to the treatment process and the presence of copper nanoparticles in the seed.

These results are strong indications that the presence of copper nanoparticles in corn seeds does not induce a great effect of toxicity to the seeds. Although there may be a negative effect of the nanoparticles due to their toxicity to plant cells, this effect should be small, to the point of impairing the physiology of the cells and generating abnormal seedlings, but not to the point of leading the seedlings to death.

In the study by Stampoulis, Sinha and White [13], it was found that seeds exposed to 100mg L-1 of Cu, germinated normally, although root growth was compromised.

In the research by Wu et al. [6], Cu nanoparticles did not inhibit the germination of tomato seeds (*Solanum lycopersicum*) in the tested doses (100-500mg L-1), data that are equivalent to this study. Less tolerant crops such as lettuce, radish and cucumber, treated with Cu suspension in water, had a 50% reduction in germination when the concentration in the suspension reached 13, 398 and 175mg L-1 of Cu respectively.

In corn seeds treated with 100mg L⁻¹ of Cu, despite the lack of effect on germination, there was inhibition of seedling growth, compared to the source of Cu [5].

Using doses of 0-1000mg L⁻¹ of copper nanoparticles in cucumber plants (*Cucurbita pepo*), after centrifugation, to remove supernatant particles during the germination period, they observed a significant increase in biomass compared to plants grown in non-centrifuged solution, suggesting a phytotoxic effect of nanoparticles [12].

In the germination of mung beans and wheat in a concentration of 1000mg L⁻¹, but they reduced root growth [17]. With soybean seeds (*Glycine max*) and chickpeas (*Cicer arietinum* L.) exposed to the suspension of Cu nanoparticles (0-2000mg L⁻¹) in a Petri dish germination test, they also did not have an inhibitory effect on germination, although root growth has been completely inhibited [14].

The analysis of variance did not reveal a significant effect ($p > 0.05$) of the hybrids in relation to the variable response percentage of normal seedlings (Figure 5).

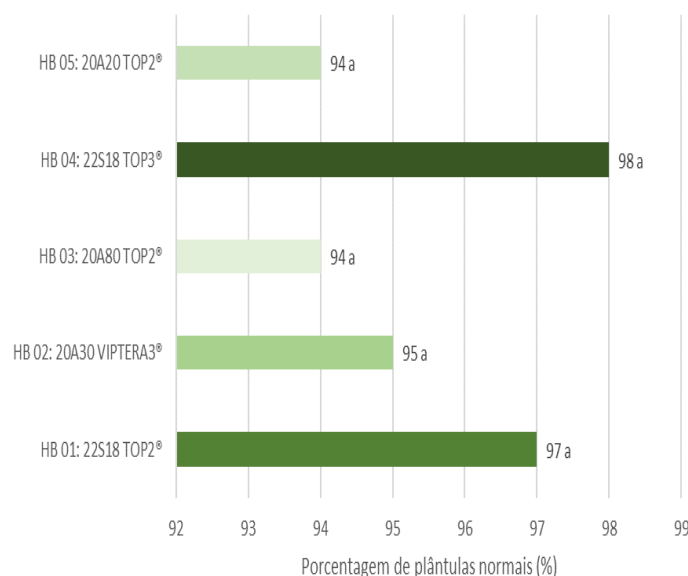


Fig.5 - Percentage of normal seedlings of the experiment in relation to the hybrid factor (São José do Cedro, SC - Crop 2018/2019)

Means followed by the same letter do not differ significantly by Tukey's test ($p \leq 0.05$)

Source: prepared by the author.

The analysis of variance revealed a significant effect ($P \leq 0.05$) of the factor of doses of nanoparticles of

Cu in relation to the variable response speed of germination, that is, there is a mathematical model that explains the influence of variable X (doses of nanoparticles of Cu) in relation to variable Y (germination speed) (Figure 05).

Corn hybrids showed an average of 95.6% in relation to normal seedlings, not expressing a significant difference between them.

It can be seen that all tested hybrids have germination values above the commercialization standard, which is 80% established by the Ministry of Agriculture, Livestock and Supply in Normative Instruction 45 [15], thus guaranteeing the necessary quality for the establishment of culture in the countryside.

3.2 Germination speed

The analysis of variance revealed a significant effect ($p \leq 0.05$) of the factor of doses of nanoparticles of Cu in relation to the variable response speed of germination, that is, there is a mathematical model that explains the influence of variable X (doses of nanoparticles of Cu) in relation to variable Y (germination speed) (Figure 6).

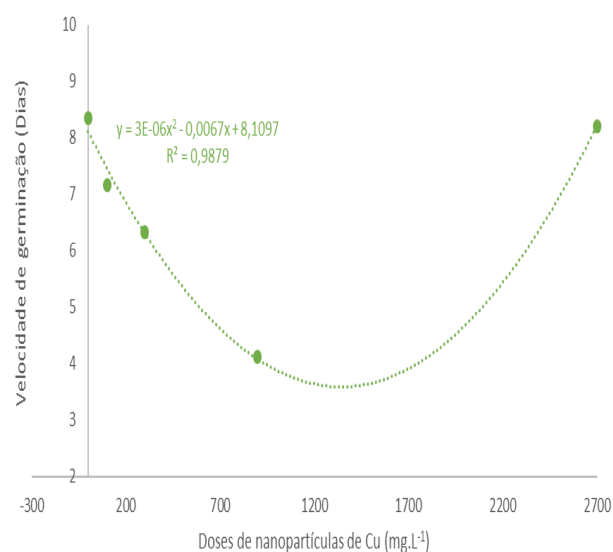


Fig.6 - Germination speed of the experiment in relation to the factor of doses of Cu nanoparticles (São José do Cedro, SC - Safra 2018/2019)

Source: prepared by the author.

As shown in Figure 06, it is noticed that there was a cause and effect relationship between the variable nanoparticles doses and the variable germination speed, that is, the doses of Cu nanoparticles influenced 98.79% in the germination speed, respectively, presenting a quadratic behavior.

From the 4th day of the germination process, it can be observed that all seeds germinated, that is, the nanocopper did not harm the process, with emphasis on the dose of 900 mg L⁻¹ that demonstrated greater effectiveness in the vigor of the seedlings.

The results indicate an optimum value for the seed treatment time and for the concentration of copper nanoparticles in the reaction medium. The longer the seed treatment time and the higher the concentrations in the reaction medium, it is possible to incorporate higher amounts of nanoparticles to the seeds without the treatment. Thus, the increase in normal seedlings is due to the presence of nanoparticles available for the seed inside.

The analysis of variance did not reveal a significant effect ($p > 0.05$) of the hybrids in relation to the germination speed response variable (Figure 7).

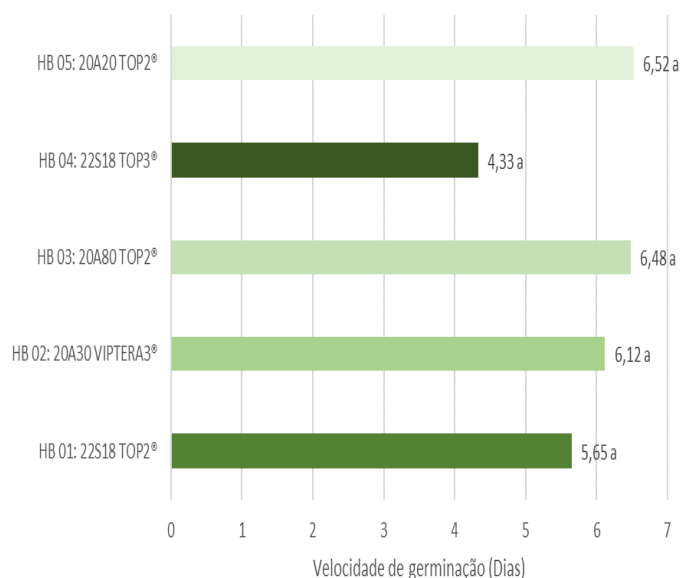


Fig.7 - Germination speed of the experiment in relation to the hybrid factor (São José do Cedro, SC - Safra 2018/2019)

Means followed by the same letter do not differ significantly by Tukey's test ($p \leq 0.05$)

Source: prepared by the author.

The presence of copper nanoparticles in the seeds did not affect the germination speed by hybrid. This indicator shows that nanoparticles do not interfere with the metabolic mechanisms of seeds in the germination phase.

IV. CONCLUSION

The copper nanoparticles did not have a negative influence on germination or on the speed of this process,

on corn seeds, since if there is a toxic effect it is small and will not compromise the metabolism of the seed. Likewise, the availability of copper for corn plants should occur at future stages of development, that is, vegetative and reproductive.

DECLARATIONS

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Conflicts of interest: The authors declare that there are no conflicts of interest

REFERENCES

- [1] Peske ST, Baudet L (2012) Seed Processing. In: Peske, S. T.; Villela, F. A.; Meneghello, G. E.; Seeds: Scientific and Technological Foundations. 3. ed., Pelotas: Ed. Universitária / UFPel, p. 457.
- [2] Souza AAB, De (2018) ZnO and CuO nanoparticles: Physiological effects on cowpea plants (*Vigna unguiculata*). 91 f., 2018. Thesis (PhD in Soil Science) - Federal University of Pernambuco, Recife, PE.
- [3] Morales-Diaz AB (2017) Application of nanoelements in plant nutrition and its impact on ecosystems. Advances in Natural Sciences: Nanoscience and Nanotechnology, Bristol, vol. 8, p. 13.
- [4] Zhao L. et al (2013) Influence of CeO₂ and ZnO nanoparticles on cucumber physiological markers and bioaccumulation of Ce and Zn: A life cycle study. Journal of Agricultural and Food Chemistry, Washington, v. 61, p. 11945-11951.
- [5] Wang X. et al (2016) Zinc oxide nanoparticles affect biomass accumulation and photosynthesis in Arabidopsis. Frontiers in Plant Science, Lausanne, v. 6, No. 1243.
- [6] Wu SG. et al (2012) Phytotoxicity of metal oxide nanoparticles is related to both dissolved metals ions and adsorption of particles on see surfaces. Journal of Petroleum and Environmental Biotechnology, Beijing, v. 3, n. 126.
- [7] Pokhrel LR, Dubey B (2013) Evaluation of developmental respondents of two crop plants exposed to silver and zinc oxide nanoparticles. Science of the Total Environment, Amsterdam, p. 321-332.
- [8] Verdi N L (2019) Agronomic aspects in corn hybrids submitted to seed treatment with copper nanoparticles. Chapecó, 2019. 82f. Dissertation (Master in Technology and Innovation Management). Community University of Chapecó Region.
- [9] Brazil. Ministry of Agriculture, Livestock and Supply (2011) Normative Instruction No. 60, of December 22, 2011. Diário Oficial da União, DF, December 23. P. 3, Section 1.
- [10] Edmond JB, Drapala WJ (1958) The effects of temperature, sand, soil, and acetone on germination of okra seeds. Proceedings of the American Society for Horticultural Science, v. 71, n. 5.

- [11] Ferreira, DF (2011) Sisvar: a computer system for statistical analysis. *Science and Agrotechnology*. v. 35, n. 6, p.1039-1042.
- [12] Stampoulis D, Sinha SK, White JC. Assay-dependent phytotoxicity of nanoparticles to plants. *Environmental Science and Technology*, Washington, v.43, p.9473-9479, 2009. Available at: <https://doi.org/10.1021/es901695c>.
- [13] Lee K, Kim BH, Lee C. Occurrence of Fusarium mycotoxin beauvericin in animal feeds in Korea. *Animal Feed Science and Technology*, v.157, n.3-4, p.190-194, 2010.
- [14] Adhikari T, Kundu, S Biswas, AK Tarafdar, JC Rao, AS. Effect of copper oxide nanoparticles on seed germination of selected crops. *Journal of Agricultural Science and Technology A*, Tehran, v.2, p.815-823, 2012.
- [15] Mapa. Ministry of Agriculture, Livestock and Supply (2013) Rules for Seed Analysis, p. 31.